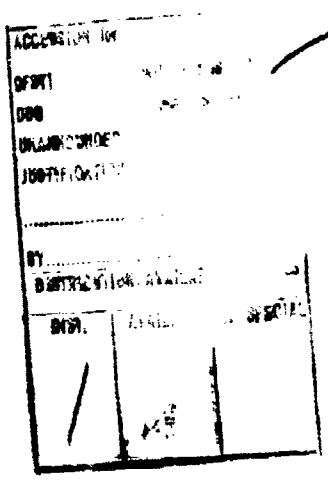
THE LEVEL OF IRON IN THE BLOOD AND LIVER OF RODENTS WITH DIVERSE INFECTIOUS SENSITIVITY TO PLAGUE

Translation No. 1610

June 1965

U. S. ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND



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THE LEVEL OF IRON IN THE BLOOD AND LIVER OF RODENTS WITH DIVERSE INFECTIOUS SENSITIVITY TO PLAGUE

/Following is the translation of an article by L. A. Avanyan, N. S. Glazko and T. M. Sosnikhina, Scientific Research Antiplague Institute for Kavkaz and Zakavkaz, appearing in the Russian-language periodical Trudy Armyanskoy Protivochumnoy Stantsii (Trudy of the Armenian Antiplague Station) 1964, No 3, pages 121-133. Translation performed by Sp/7 Charles T. Ostertag Jr./

It has been established by the investigations of numerous authors, both local as well as foreign, that various species of rodents display a diverse degree of infectious sensitivity to plague, that a different sensitivity to plague is displayed by rodents of the same species which are residing under diverse ecological conditions, and that the time of year and age of the rodents also influenced the course of plague infection. These species, seasonal and age differences in the sensitivity are observed not only in the laboratory but also in wild rodents, including those which are natural carriers in natural foci of plague.

I. S. Tinker and Ye. N. Aleshina (1955) established that little susliks which resided in various landscape-ecological conditions displayed a various sensitivity to plague. Ye. S. Biryukova (1957), M. I. Levi and co-authors (1959) experimentally proved that midday gerbils from the right bank of the Volga were more sensitive to plague than midday gerbils which resided on the left bank of the Volga. U. A. Mamed-Zade and co-authors (1957) point out that the infectious sensitivity to plague of redtailed gerbils varied in different years and various seasons. M. F. Shmuter and co-authors (1957, 1958) showed that jerboas (emuranchicks and tarbagans) are more sensitive to plague than white mice. In the tests by L. S. Malafeyevaya (1959) the infectious sensitivity of Tamarisk gerbils to plague was considerably higher than that of midday gerbils. A. K. Akiev and I. I. Vologina (1960) point out that the sensitivity of great gerbils to plague in the early spring months is greater than in the spring months. M. Balpazar and co-authors (1960) established that out of four species of gerbils in the Kurdistan plague foci the Vinogradov gerbils and the little Asian gerbils are more sensitive to plague than the Persian and redtailed gerbils. A. A. Levina (1960), while studying the mechanism of preservation of the plague causative agent in natural foci during inter-epizootic seasons and years, points out that the redtailed gerbils are more sensitive than the great gerbils. On the basis of literary data, A. G. Kratinov (1957) considers that guinea pigs, white mice, and white rats, belong to the group of rodents which are highly sensitive to plague, and that common and social voles belong to the group of rodents which are highly sensitive to plague. A. G. Kratinov and co-authors (1960, 1961) point out the species differences in the sensitivity of rodents to plague toxin and the change in the content of ascorbic acid in the organs of these rodents during plague intoxication.

This list of literary data, which is far from complete, indicates that the infectious sensitivity of various species of rodents to plague bears not only a species but also a seasonal nature.

The nature of these species, seasonal and age differences in the resistance and sensitivity to the plague causative agent have not been established up to the present time, though the resolving of this important problem may be of significant interest for the comparative pathology of plague, for an understanding of the species differences in the pathogenesis of plague, and for clearing up the role of infectious sensitivity of the carriers and the dynamics of the course of plague epizootics and in the maintenance of natural foci of this infection. There are all the bases to assume that the infectious sensitivity or resistance of rodents is determined by a complex of factors.

In the complex process of the interrelationship of the plague microbe and its carriers a very important role is played by two factors: a) Factors which cause or promote the multiplication of the microbe in the organism of the host; b) Factors, found in the organs, tissues and cells of the hosts which cause the depression or death of the causative agent. In the final calculation the qualitative and quantitative relationship of these factors also determine the possibility of the infection developing so much as to appear in the form of a disease (R. Dyubo).

The present report deals with iron, which promotes the multiplication of the plague microbe invitro and invivo and increases its virulence in experiments on guinea pigs and white mice (Jackson and Burrows, 1956; Avanyan and Gubina and Ivanova, 1963; Klassovskiy and Terentyeva, 1963).

L. A. Avanyan with co-authors (1963) showed that in virulent strains of the plague microbe, the ability to assimilate iron on nutrient media is greater than in avirulent strains.

Subsequently, with the aim of clearing up the role of iron in the pathogenesis of plague, we studied its content in various species of rodents. In the first investigations (Avanyan, Glazko, 1962), we detected that in the midday gerbils from the right bank, which are highly sensitive to plague, the content of iron in the blood was greater than in gerbils from the left bank which were mildly sensitive to plague. Subsequently we also investigated the fluctutations in the level of iron in certain organs of the reticuloendothelial system, particularly in the liver.

E. V. Domaradskiy with co-authors (1960) note that the leading role in combating intoxication of any origin undoubtedly belongs to the liver and that in all probability during plague the outcome of the disease is determined to a considerable degree by the functional condition of this organ. It is known that the liver is the basic organ regulating the level of iron in the blood and other tissues and cells of the organism. A. Mazur, S. Grin and A. Karlston (1960) point out that the stronger the oxidizing reaction in the liver then the greater is its capability to accumulate a greater amount of

iron in the form of ferritin. Here an important role is played by adenosine triphosphate and the ascorbic acid of the liver, and also the amount of beta-globulins of the blood serum of the animal. These substances guarantee the transfer of iron from the blood to the liver.

L. Goldberg, L. Martin and A. Batchelor (1960) prove in a great deal of experimental material that with an increase of iron in the liver in several species of rodents there is an increase in the activity of a number of enzymes, including acidic phosphatase, and the latter activates the work of liver macrophages.

L. Kheylmeer (1957, 1959), Kheylmeer and F. Veler (1961) consider that the capability of the liver to deposit iron in the form of ferritin and haemosiderin increases the defensive function of the organism against infections, particularly intestinal. K. H. Matsutani (1959) points out that a destruction of the function of the liver is accompanied by an increase in the content of iron in the blood serum and also a significant increase of non-hemin iron in the spleen and kidneys. Here also there is a significant change in the protein fraction of the liver and there is a decrease in the albumin and ferritin. G. Shandl, G. Inman, R. Simonons and B. Allen (1959) write that to the greatest degree the iron binding protein of the plasma binds iron when it is incompletely saturated; when it is completely saturated iron behaves as an ion.

S. Jackson, B. Morris (1961), when incubating various strains of the plague microbe on the normal blood serum of man and white mice, studied the influence of several cations (Co, Ni, Cu, Fe, Mn, Zn, Ca and Mg) and established that only with the addition of iron did the act of growth take place and the accumulation of a large amount of bacteria. K. H. Martin (1962) established experimentally that the bacteriostatic capability of the serum from patients with aggamaglobulinaemia against Bac. subtilis BPCC 663 is overcome by the addition of iron. The authors point out that in the blood serum the more transferritin which is free from iron (beta-globulin) then the greater its bacteriostatic capability. A. B. Gulenko (1962), in imparting a prognostic significance to the investigation of non-hemin iron in the blood plasma during infectious diseases, writes that an accumulation of iron in the retinculoendothelial system in the opinion of Kheylmeer, Kayberling and others, is connected with the non-specific defensive system of the organism. In the course of an infectious disease there is an increase in the activity of the reticuloendothelial system, and this leads to an increase of cellular respiration, for the realization of which it is necessary that there be an increase in the production of the enzyme ferrocystine, a component part of which is iron. If it is considered that to the same degree an accumulation of iron in the reticuloendothelial system determines the non-specific defensive function of the organism then it can be proposed that a lowering of the capability of the organism for this reaction will promote an increase of sensitivity to infection.

All these facts impelled us to engage in the study of the status of iron exchange in rodents - the carriers of plague.

Methods and Materials of Investigation.

Basically, we have described the methods of operation earlier (Avanyan, Glazko, 1962). The iron in the blood and liver was determined in the mineralyzate by the orthophenontrolin method following the moist combustion of the suspensions [illegible]. The amount of iron was calculated for 100 grams of blood and for 100 grams of raw liver tissue. In order to exclude iron which was found in the liver due to the blood, we, in parallel-suspensions of the liver, determined the volume of blood based on the method of A. V. Tkachenko (1963).

The samples for analysis for emuranchicks, right bank and left bank midday gerbils and for common voles of the Stavropolskiy Kray were taken in the [illegible] of the Institute at Stavropol, and for the remaining rodents - at [illegible].

Results of the Investigation and Discussion.

The results of our investigations, which are presented in table 1, show that the level of iron in the blood and liver fluctuates both within each group of animals as well as in rodents of various species.

Along with this, the results of the tests show that for rodents which are less sensitive to plague (common voles) there is more iron in the liver than for rodents which are more sensitive to plague (guinea pigs, white mice, right bank midday gerbils). The amount of iron in the blood of such plague-sensitive rodents as the emuranchicks and the right bank midday gerbils were noticeably higher in comparison with the others.

In the first of our observations (Avanyan, Glazko, 1962), it appeared to us that when the level of iron in the blood of rodents was greater, then they were probably more sensitive to plague. However, further observations showed that the absolute amount of iron in the blood or in the liver did not always serve as an estimate for determining the resistance or sensitivity to plague of this or that species of rodents.

If only the absolute concentration of iron in the blood is considered (table 1), then the guinea pigs should be regarded to the group of rodents which are mildly sensitive to plague, that is for guinea pigs the content of iron in the blood is not high, although the iron in their livers was less in comparison with other species of rodents. If the amount of iron in the liver is taken as the basis for evaluating resistance then such plague-sensitive rodents as emuranchicks should be in the group of resistant animals, since the level of iron in their livers is high, although the amount of iron in the blood for these rodents is greater.

[illegible] on the sensitivity of rodents to plague, on the role of iron in the non-specific protective reaction of the organism against infection, and also the results of our investigations on the content of iron in the blood and organs for species of rodents which are different in their sensitivity to

plague, made it possible for us to consider that when determining the role of iron in the infectious sensitivity of rodents to plague apparently it is necessary to consider not only its absolute content in the blood or in the liver, but also the ratio of these indices, that is the relationship of the amount of iron in the blood to the amount of iron in the liver -

$$\frac{\text{Iron in the blood}}{\text{Iron in the liver}} .$$

We consider that this relationship in some degree shows both the level of the organism's ability to accumulate iron in the liver (in the organs and tissues), as well as the level of labile (non-hemin) forms of iron, which are formed in the blood (in the serum) when there is a high content of it and may block the bacteriostatic properties of the serum. We propose that the higher this index is then the less is the ability of the rodent to deposit iron and it is possible the more sensitive the organism of the rodent to plague infection. If the species of rodents investigated by us are taken on the basis of this index (i.e., the relationship of the iron in the blood over the iron in the liver) and placed in descending order then the impression is created that in actuality the rodents which are most sensitive to plague are located in the upper part and the rodents which are least sensitive in the lower part of the table (table 1). It must be noted that the factual data obtained by us does not fully satisfy this assumption. For example, based on the data of bacteriological investigations (data from the literature) the redtailed gerbils are more sensitive to plague than the great gerbils. However, in the table the great gerbils are located higher than the redtailed gerbils. Such a nonconformity between the data of bacteriological investigations and our data is observed between the emuranchicks and white mice, i.e., the white mice are located higher in the table than the emuranchicks. If it is considered correct that white rats belong to the group of rodents which are sensitive to plague then again our data contradicts the data of bacteriological investigations.

The use of an insignificant number of animals in the tests and the individual biochemical or bacteriological investigations cannot serve as criteria for clearing up such a complex process as the non-specific protective reaction of an organism against infection. We are far from the notion of attributing this complex phenomena to only one element - iron. Without a doubt, the organism possesses a complex of factors which take part in its defensive reaction against plague infection. The purpose of our investigations is to stress the important role of iron in this process and the necessity for expanding the biochemical investigations in this direction.

Table 2 presents the significance and the difference of the average values in the amount of iron in the blood and liver for several species of rodents which are sensitive and moderately sensitive to plague on the basis of the indices presented in table 1.

The evaluation of the significance of the difference in the average values was calculated according to the formula:

$$x_1 - x_2 > t_{ps} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \quad ,$$

utilizing the tables of Styubent-Fisher (I. P. Ashmarin and A. A. Vorobyev, 1962, page 31).

The diverse ecological, seasonal and other conditions in the life of rodents often make it difficult to obtain accurate, comparable and statistically reliable data for chemical analysis. Based on the data of G. P. Bondarenko (1962), mobile forms of iron in the soil fluctuate both during the year as well as in various years.

A. Thompson and A. Reyvin (1959), G. Klavins, P. Kleniey and N. Kaderman (1962) point out that the absorbability of iron in the organism depends strongly on the composition of the food, and according to the data of S. Reynfark, K. Losono and G. Valdevyes (1959) - on the geographical conditions.

Our data are preliminary. Subsequent investigations will introduce many corrections and adjustments in our concept concerning the role of iron in the infectious sensitivity of rodents to plague.

Conclusions.

The data obtained by us makes it possible to consider that in all probability in rodents which are comparatively mildly sensitive to plague the ability to deposit iron in the liver is greater than the rodents which are highly sensitive. At the same time, the index of the relationship of iron in the blood to iron in the liver in highly sensitive rodents is greater than in mildly sensitive rodents.

However, the data obtained by us cannot be unconditionally passed on as a regularity for rodents of all these species investigated by us.

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Table 1

Content of iron in the blood and liver in certain rodents

Rodents	Number of animals	Iron in mg%		Index of relationship of blood iron to liver iron	Place where captured
		Blood M ± m	Liver M ± m		
Guinea pigs	22	45.16±0.98 (36.3--52.9)	4.24±0.72 (1.7--10.0)	10.7	Stavropol, Laboratory
White mice	10	52.82±2.1 (40.9--63.3)	5.56±1.31 (3.66--14.45)	9.4	Stavropol, Laboratory
Emuranchiks (awake)	10	77.84±2.5 (65.9--89.1)	12.6±1.87 (7.6--17.58)	8.8	Stavropol Kray Achinkulakskiy R.
Midday gerbils, right bank	20	61.37±1.68 (48.7--82.2)	9.24±1.02 (8.6--18.01)	6.6	Kalmytskaya ASSR, Chernozemelskiy State Farm
Great gerbils	19	39.98±1.15 (29.7--49.2)	7.65±0.70 (7.3--15.77)	5.2	Turkmenskaya SSR, Ashkhabad, Krasnovodsk
Red-tailed gerbils	10	46.21±1.64 (42.2--57.8)	9.45±1.08 (8.6--16.23)	4.9	Turkmenskaya SSR, Krasnovodsk
Midday gerbils, left bank	20	54.32±1.52 (39.5--68.5)	11.41±1.24 (7.3--19.64)	4.8	Astrakhanskaya O. Dosang
Common voles	20	40.49±1.14 (27.5--50.3)	12.64±1.06 (11.3--26.14)	3.2	Astrakhanskaya O. Dosang R.
Common voles	19	43.73±1.74 (27.4--56.3)	14.55±1.04 (8.2--23.47)	3.0	Azerbaydzhan SSR, Nakhichevanskaya ASSR, Shakhbuzskiy R.
European susliks (awake)	10	49.21±0.90 (44.3--54.6)	16.89±1.67 (11.2--26.45)	2.9	Armenian SSR, Talinskiy R.
White rats	15	49.27±1.97 (38.0--59.1)	17.4±1.68 (14.3--28.29)	2.8	Stavropol, Laboratory
Common voles	10	48.71±1.4 (41.2--56.7)	17.60±3.35 (11.1--40.33)	2.7	Stavropol Kray, Kardonik

O. = Oblast; R = Rayon.

Table 2

Reliability of the differences of the average values in the content of iron in the blood and liver in several rodents (based on the data of table 1).

Species of Rodents	Blood	Liver
Guinea pigs Common voles (Armenian SSR)	$P<0.01$	$P<0.01$
Guinea pigs Great gerbils	$P<0.05$	$P<0.01$
White mice Common voles (Armenian SSR)	$P<0.01$	$P<0.01$
White mice Common voles (Azerbaydzhan SSR)	$P<0.01$	$P<0.01$
White mice White rats	$P<0.05$	$P<0.01$
Emuranchiks Common voles (Stavropolskiy Kray)	$P<0.01$	$P>0.05$
Midday gerbils, right bank European susliks	$P<0.01$	$P<0.01$
Midday gerbils, right bank Midday gerbils, left bank	$P<0.01$	$P>0.05$
Redtailed gerbils Common voles (Armenian SSR)	$P<0.05$	$P>0.05$